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Green Oxidation of Starch Using Ozone: A Comparative Study on Rheological Properties

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Abstract

Pea starch, often obtained as a by-product of pea protein isolation, is increasingly available and economically attractive. Consequently, the industry is seeking new applications of pea starch, both in its native and modified forms. This paper highlights the topic of pea and potato starch oxidation with ozone in aqueous suspension and evaluates the effect of process time, retention volume and solids content on pasting, texture, and flow behavior, benchmarking against a commercial hypochlorite-oxidized product. Moreover, obtained preparations were studied for their molecular mass distribution and hydrodynamic parameters. It was found that the oxidation of both potato and pea starch with ozone in an aqueous suspension is an effective method of obtaining this type of starch preparations. The extent of modification was dependent on all variables considered in the research. The depolymerization of both starch varieties progressed gradually, but the oxidation effects were more noticeable for potato starch compared to pea starch, which was found to be related to the gelling characteristic of those preparations.

Keywords: modified starch; oxidized starch; pea; potato; pasting characteristics; TPA; GPC; zero waste

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1. Introduction

Starch is the most important plant polysaccharide in terms of human nutrition. However, its economic importance goes far beyond being a source of dietary energy. Above all, starch is found in products made from flour, i.e., bread, breakfast cereals or pasta. Nevertheless, in the food industry, it is widely used as stabilizer and texture forming agent. The main field of application includes sauces, soups, and mayonnaises as well as different dairy and meat products. Moreover, starch is used for non-food applications, mainly in papermaking, but also in textile, pharmaceutical or even building material industries. Starch is composed of two macromolecular fractions: amylose and amylopectin. Amylose is generally recognized as linear polymer consisting of D-glucopyranose units linked by α-(1,4) glycosidic bonds. However, a slight degree of branching has been reported. Amylopectin contains additional α-(1,6) glycosidic bonds, which create branches in the structure of the macromolecules. The properties of starch are determined by a number of structural factors depending mostly on its botanical origin. Among them, the amylose to amylopectin ratio is of key importance [1–3]. Despite the large variety of physicochemical and functional properties of the common commercially available native starches, i.e., corn, tapioca, potato,

and wheat to meet the needs of the industry, modification is necessary. Currently, the value of products from starch processing exceeds two and a half times the value of starches used in their native form. Starch, both in its native and modified form, is mainly used in the food industry. However, the value of starch in non-food application is on the level of about 50% of that in food industry [4–6].

Starch can be modified by physical, chemical, or enzymatic methods. Physical modification, on industrial scale, is mainly applied to give the starch the ability to dissolve in cold water. Enzymatic processes mostly involve hydrolysis of starch resulting in low molecular mass products such as glucose, fructose, and a wide range of syrups and maltodextrins. Nevertheless, products of transglycosidation are also available on the market. The widest range of products can be obtained through chemical modification. They are used both in the food industry as well as for non-food applications. The numerous reactions that are applied in the chemical modification of starch can be generally classified as hydrolysis, oxidation, esterification, and etherification. If multifunctional reagents are used in esterification or etherification reactions, starch undergoes cross-linking. Acid hydrolysis and oxidation are traditional methods for chemical modification of starch. While acid hydrolysis is losing its importance due to the development of enzymatic technologies, oxidation is still a popular method [7–12].

Starch oxidation can be carried out using various reagents among which the following should be mentioned: sodium hypochlorite, hydrogen peroxide, ozone, and periodates. The mechanism of the reaction as well as the structure and properties of the obtained products strongly depend on the reagent used [13,14]. The most common in the industry is the oxidation reaction employing NaOCl. This process has been known for many years, as the safety of its use for food production has been confirmed in numerous studies [15,16]. The reaction mechanism has also been deeply studied. Already in the 1950s it was established that the oxidation of the starch by sodium hypochlorite results in cleavage of anhydroglucose unit between carbon atoms 2 and 3. Then it has been determined that carbon 6 is also oxidized [17,18]. The reaction between starch and sodium hypochlorite is influenced by pH, temperature, time and of course the dose of active chlorine. The oxidation process is accompanied by depolymerisation that results in decrease in average molecular mass [13,19]. Another oxidation process that has been known for many years is the reaction with periodates. However, in contrast to hypochlorite oxidation it has not been widely used in industry. The product of this process is dialdehyde starch, which is formed through selective splitting of the bond between carbons 2 and 3 and then the oxidation of hydroxyl groups. Due to the presence of reactive aldehyde groups, dialdehyde starch reveals antioxidant and antimicrobial activity; moreover, it can serve as cross-linking agent. These features may become particularly useful in the industrial applications [13,14]. The next starch oxidizing agent, which was extensively studied, is hydrogen peroxide. However, similarly to oxidation with periodates, this technology is not widely used in the starch industry. The mechanism of reaction involves radical processes and can be catalyzed by metal ions. The interest of the researchers in this technology results from the lack of necessity of using toxic chlorine [13,20].

An emerging technology in starch modification is its oxidation by ozone. It is the most eco-friendly of all starch oxidation methods. Ozone as an oxidizing agent can be incorporated into the reaction medium in the form of a gas or an aqueous solution. Particular attention has been paid to ozonation of the most popular commercial starches, i.e., corn, wheat, potato, and cassava. However, the results presented in the literature are not repeatable and even contradictory. Regarding the reaction mechanism, it has been reported that by ozonation more carbonyl than carboxyl groups are formed and the oxidation is accompanied by cross-linking [21–28]. Above all, the potential applications of

starch modified by ozonation are studied. The possibility of production of biodegradable films is of particular interest [29–31]. This is especially important in the case of the less common starches that are not recognized as useful. This concerns especially pea starch and other originated from pulses. They reveal a restricted type of swelling characteristic, which limits their usefulness, especially in food production. Although this feature of starch isolated from pulses may be partially alleviated by modification (including oxidation). Furthermore, the high amylose content in legume starches could be recognized beneficial in terms of nutrition, as it facilitates the formation of fraction resistant to amylolytic enzymes. However, that application of starch is still less popular than was previously expected. The high amylose content can also be beneficial in some non-food applications, as it improves the film-forming properties of starch. Moreover, the availability of pea starch on the market is expected to increase as the demand for pea protein increases due to the popularization of vegetarian and vegan diets [6,32].

Taking into consideration the above the aim of the study was to develop a method for modifying starch using ozone in aqueous medium and to obtain oxidized preparations based on pea starch. The scope of the work included analysis of the impact of selected process parameters (time, ozone dose, and suspension concentration), rheological characteristics of obtained starch preparations, and determination of molecular mass distribution and hydrodynamic parameters of starch macromolecule in the solution, followed by comparative study with ozonated potato starch as well as commercial potato starch oxidized with sodium hypochlorite.

2. Materials and Methods

2.1. Starch Raw Material

Commercially available food grade starches were used as an initial working material. Native potato starch superior standard (WPPZ S.A., Luboń, Poland) and pea starch (Emsland-Stärke GmbH, Emlichheim, Germany) were subjected to ozone treatment in pilot scale ozone treatment installation. Oxidized starch (by sodium hypochlorite) LU-1404-1 (WPPZ S.A., Luboń, Poland) with carboxyl group content of 0.05% was used as reference material.

2.2. Starch Modification Procedure

Oxidation reactions of starch in the form of an aqueous suspension (20 or 30% w/w) were carried out on a pilot scale. The process was conducted in a flow-through system by means of repeated circulation in a closed circuit, and the retention time depended on the capacity of the buffer tank (retention volume). To replicate the use of technological water (as chemical modifications are performed at industrial scale) the pilot trials were run on tap water with following characteristics: pH 7.4, conductivity 820 μS/cm, and water hardness of 350 mg·L^{-1} $CaCO_3$ equivalent.

Ozone treatment reactor was assembled by ozone water generator model Atom Water 5 (Blue Planet, Wolsztyn, Poland) with ozone injection via Venturi tube, screw pump with adjustable capacity (160 L/h) and thermostated (20 °C) buffer tank with agitator with volume of 4 L (1.5 min retention time) or 40 L (15 min retention time). The declared ozone production capacity of the generator was 5.5–10 L per minute of 1–3 ppm ozonated water (300 W rated power). The measured ozone concentration in pure water at the return to the buffer tank of the assembled system was 5.4 ± 0.2 mg·L^{-1} of water. The measurements were performed using DOZ30 ozone meter (Blue Planet, Wolsztyn, Poland) in water blank (measurements in slurry were not possible due to detection interference).

Series of tests were carried out on native potato and pea starch in the form of a circulating water suspension with varying time, retention volume, and suspension concentration.

Overall, five series of experiments with constant flow rate and temperature were performed as follows:

- Potato starch treated for 1 h, 2 h, 4 h, and 6 h, suspension concentration 20%, 15 min retention time (ozone dose of 0.11 ± 0.00; 0.22 ± 0.01; 0.43 ± 0.02 and 0.65 g ± 0.02 per 1 kg of starch, respectively).
- Pea starch treated for 1 h, 2 h, 4 h, and 6 h, suspension concentration 20%, 15 min retention time (ozone dose of 0.11 ± 0.00; 0.22 ± 0.01; 0.43 ± 0.02 and 0.65 g ± 0.02 per 1 kg of starch, respectively).
- Potato starch treated for 6 h, suspension concentration 20%, 1.5 min retention time (ozone dose of 6.48 g ± 0.24 g per 1 kg of starch).
- Pea starch treated for 6 h, suspension concentration 20%, 1.5 min retention time (ozone dose of 6.48 g ± 0.24 g per 1 kg of starch).
- Potato starch treated for 6 h, suspension concentration 30%, 1.5 min retention time (ozone dose of 4.32 g ± 0.16 g per 1 kg of starch).

2.3. Proximate Composition of Starch

Starch moisture content was determined by gravimetric analysis according to PN-EN ISO 1666:2000 [33]. Starch protein content was determined using Kjeldahl method according to PN-EN ISO 3188:2000 [34]. Starch content was determined using Ewers polarimetric method according to PN-EN ISO 10520:2002 [35]. Ash content was determined by incineration according to PN-EN ISO 3593:2000 [36]. Carboxyl group content was determined by titration method after sample demineralization according to PN-EN ISO 11214:2001 [37].

2.4. Rheological Characterization of Starch

2.4.1. Pasting Characteristics

Pasting characteristics of starch aqueous suspensions were recorded with a Brabender Viscograph (Duisburg, Germany) according to methodology described in detail previously by Lewandowicz and coauthors [3]. In brief, suspensions were heated/cooled at 1.5 °C per minute rate within a 25–92.5–25 °C temperature ramp, incubation period of 20 min and measuring range of 700 cm was used. Suspension concentration was adjusted to perform analyses at a maximum concentration that does not exceed 2000 BU (Brabender units) at any stage of the analysis. Effectively, potato starch was analyzed at a concentration of 5%, whereas for pea starch, 7.5% suspension was used. Obtained pasting curves were analyzed in terms of gelatinization temperature °C, peak viscosity BU, breakdown BU, setback BU, end of holding period viscosity BU, and final viscosity BU.

2.4.2. Texture Profile Analysis

Universal texture profile of starch pastes were determined with assistance of TA-XT2 texturometer (Stable Micro Systems, Godalming, UK) fitted with 35 mm cylindrical probe and 5 kg load cell. The detailed procedure was described previously by Lewandowicz and coauthors [3]. In brief, samples were penetrated at a depth of 20 mm with 0.5 mm/s speed. Trigger force value was set to 5 g. Measurements were performed on starch pastes prepared with the assistance of Brabender viscograph (previous step), immediately after preparation.

2.4.3. Rheological Properties

Rheological properties of starch pastes obtained at stage 2.3.1. were determined immediately after preparation using a RotoVisco1 rotational rheometer using coaxial measurement geometry Z20 DIN with default 4.2 mm gap (Haake Technik GmbH, Vreden, Germany) Flow curves were recorded within 0.1–600 s^{-1} shear rate according to procedure described previously by Lewandowicz and coauthors [3]. Obtained data were fitted to

the Ostwald de Waele model. Moreover, the area of hysteresis loop of thixotropy was calculated based on the difference between upwards and downwards flow curve (negative values indicates anti-thixotopic behavior). Data collection and calculations were made using RheoWin 3.61 software.

2.5. Molecular Characterization of Starch Using SEC with Triple Detection

SEC equipment from Malvern, TX, USA, equipped with triple detection (Viscotek 305 TDA) was used to separate samples. A dual-cell refractometer (RI), viscometer (Vis), and light scattering detectors (including low-angle light scattering, LALS, and right-angle light scattering, RALS) were used simultaneously. SEC analysis was performed using a SEC column (PSS GRAM series, 3000 Å, 8 × 300 mm) with a guard column (Polymer Standard Service GmbH, Mainz, Germany).

Starch samples were dissolved in DMSO at 70 °C for 24 h with gentle stirring using a Reacti-Therm (Thermo Fisher Scientific, Waltham, MA, USA). The concentration of the samples was 1.5 mg/mL. The samples were not filtered prior to analysis. The injection volume was 50 µL. The flow rate of DMSO was 0.3 mL/min at 70 °C. The detector response factors were determined using the pullulan standard (113,000 g/mol). The refractive index (RI) of the solvent was 1.4595 [38]. Calculations were performed using OmiSEC 4.7 software (Malvern, TX, USA), assuming a refractive index increment (dn/dc) of 0.0659 mL/g for starch in DMSO [39]. The absolute molecular weights were calculated. The following parameters were computed from three independent samples' preparation of the sample: M_n—number average molar weight; M_w—weight average molar weight; M_z—z-average molar weight; M_w/M_n—polydispersity index, R_h—hydrodynamic radius, R_g—radius of gyration. SEC chromatograms (signals from the refractive index detector) are presented in Figures S1 and S2.

2.6. Statistical Analysis

All analyses were performed in triplicate (unless otherwise stated) on individually prepared samples of one production batch, and the results are presented as mean value ± standard deviation. Experimental data were studied using a one-way analysis of variance and Tukey's post hoc test, differences with $p < 0.05$ were considered significant. Moreover, principal component analysis (PCA) was performed based on the correlation matrix based on rheological characterization data. The statistical analyses were performed using Statistica 13.3 software (TIBCO Software Inc., Palo Alto, CA, USA).

3. Results

3.1. Proximate Composition of Starch

Modern starch isolation technologies that are used at industrial scale can provide preparations of very high purity. That was the case of both starch varieties subjected to ozone treatment in this study. Nevertheless, their macro and micro-constituent content varied significantly due to botanical origin (Table 1). Pea starch has significantly lower moisture content than potato starch, which may be attributed to differences in the crystalline structure. Potato starch typically has a type B crystal structure, which is characterized by the higher proportion of water in the crystal lattice. Pea starch preparation had also approximately five times higher protein content, which is the result of more challenging separation process observed for legume starch sources. However, this isolation impurity did not affect significantly the measured starch content, which was slightly but significantly higher than for potato starch preparation. This observation was presumably the result of a high amount of mineral impurities as indicated by ash content. This phenomenon is typical and associated with the presence of phosphate groups in potato starches, which have the

ability to bind metal ions through ionic bonds. Lastly, both starches differed significantly in efficiency of carboxyl group formation during ozonation process. Although after the most extensive treatment with ozone both preparations were characterized by carboxyl group higher than reference LU-1404-1 (which was 0.05%), the efficiency of modifying group formation was three times faster in case of potato starch. Nevertheless, the extent of modification was still at a level of purity specification for food grade modified starches, which in extreme cases can reach values of 1.1% [15]. Nevertheless, for commercially available preparations usually lower values are reported 0.03–0.11% [40]; 0.04–0.50% [41].

Table 1. Proximate composition of native starches subjected to ozone treatment.

Parameter	Potato Starch	Pea Starch
Moisture [%]	19.1 ± 0.3 [b]	12.0 ± 0.2 [a]
Protein [%]	0.17 ± 0.01 [a]	0.88 ± 0.05 [b]
Starch [%]	96.9 ± 0.2 [b]	97.8 ± 0.1 [a]
Ash [%]	0.38 ± 0.03 [b]	0.10 ± 0.03 [a]
Carboxyl groups [%] (after most extensive ozonation)	0.33 ± 0.01 [b]	0.11 ± 0.00 [a]

Mean value of two replicates ± standard deviation; values marked with the same letter do not differ significantly.

3.2. Rheological Studies of Potato Starch

3.2.1. Influence of Oxidation Time

To analyze the effect of oxidation time, a 20% starch suspension in large circulation system (40 L buffer tank) was studied. A series of tests was conducted, and analytical samples of approximately 1 L were taken from the circulation system at selected time intervals. The pasting characteristics parameters are presented in Table 2, whereas pasting curves are shown in Document S3.

Table 2. Pasting characteristics of 5% potato starch suspensions subjected to different durations of ozone treatment.

Starch	Pasting Temperature (°C)	Maximum Viscosity (BU)	Breakdown (BU)	Setback (BU)	End of Holding Period Viscosity (BU)	Final Viscosity (BU)
Native	61.9	1879	1303	506	570	1082
Oxidized 1 h	63.1	1366	841	624	525	1151
Oxidized 2 h	62.5	1435	962	547	473	1021
Oxidized 4 h	62.5	1292	963	393	328	724
Oxidized 6 h	63.2	1176	933	284	243	528
Commercial	63.8	525	221	171	304	475

Mean value of three replicates; differences below 0.2 °C or 30 BU are considered not significant.

The swelling characteristics of native potato starch and its derivatives were typical for starches derived from tubers (high type), i.e., they were characterized by a high increase in viscosity at the beginning of gelatinization, a rapid breakdown during holding stage, and pronounced setback during the cooling stage. A commercial preparation of oxidized starch (E 1404), also obtained from potato starch and modified using sodium hypochlorite (LU1404-1), had identical gelatinization characteristics. The oxidation process caused a slight increase in the gelatinization temperature of the tested preparations, but without a clear trend and at a level that was insignificant from a technological point of view, with the extreme difference being only 1.3 °C. The commercially oxidized starch preparation also had a slightly higher gelatinization temperature but similarly at a level that was insignificant from a food technology perspective. Under the influence of ozone and with

longer modification time, a progressive decrease in all gelatinization parameters were observed, including peak viscosity, breakdown, setback, viscosity at the end of holding period, and final viscosity. The only exception was observed for starch subjected to the shortest oxidation process (1 h), whose final viscosity was slightly higher than that of the initial product. This was associated with an increase in viscosity during the cooling phase (setback), which may be attributed to the gelation process that is typical of oxidized starches.

The preparations obtained after 4 and 6 h of oxidation were characterized by similar values of final viscosity and viscosity in the final phase of holding period, to the commercial preparation (LU1404-1). However, the course of gelatinization of the preparations obtained with the use of ozone was more rapid, which was reflected in the high values of the parameters such as peak (maximum) viscosity, breakdown, and setback.

In the next stage of the study, the oxidized potato starch pastes obtained in the Brabender viscograph were subjected to universal texture profile analysis (Table 3). The parameters that differentiated the tested starch pastes the most were hardness, adhesiveness, and gumminess. The latter, which is a secondary parameter representing the product of hardness and cohesiveness, varied mainly due to changes in hardness. The course of changes in the texture profile of the tested starch pastes was similar to the data obtained from the analysis of the pasting process, i.e., a decrease in the absolute values of the tested texture profile parameters was observed. The only exception (as in the case of the pasting process) was observed for the paste obtained from starch subjected to the shortest oxidation process, for which an increase in hardness, adhesiveness, and gumminess was observed. This preparation was also the most similar to the commercial product LU1404-1. The only difference between the two pastes considered was the adhesiveness parameter. This characteristic is usually negatively perceived by consumers, but it can be a desired property in other non-food applications.

Table 3. Texture profile of 5% potato starch pastes obtained from preparations subjected to different durations of ozone treatment.

Starch	Hardness (N)	Adhesiveness (N·s)	Cohesiveness (-)	Springiness (-)	Gumminess (N)
Native	0.45 ± 0.02^{a}	-0.10 ± 0.02^{ab}	0.73 ± 0.00^{d}	0.97 ± 0.00^{cd}	0.33 ± 0.02^{bc}
Oxidized 1 h	0.58 ± 0.01^{b}	-0.40 ± 0.03^{d}	0.68 ± 0.01^{ab}	0.94 ± 0.00^{a}	0.39 ± 0.00^{c}
Oxidized 2 h	0.43 ± 0.01^{a}	-0.14 ± 0.01^{bc}	0.72 ± 0.00^{cd}	0.96 ± 0.00^{bc}	0.31 ± 0.01^{ab}
Oxidized 4 h	0.43 ± 0.00^{a}	-0.08 ± 0.01^{ab}	0.70 ± 0.00^{bc}	0.97 ± 0.00^{cd}	0.30 ± 0.00^{ab}
Oxidized 6 h	0.41 ± 0.00^{a}	-0.04 ± 0.02^{a}	0.70 ± 0.02^{bc}	0.98 ± 0.01^{d}	0.29 ± 0.01^{a}
Commercial	0.57 ± 0.07^{b}	-0.21 ± 0.07^{c}	0.67 ± 0.00^{a}	0.95 ± 0.00^{ab}	0.38 ± 0.05^{c}

Mean value of three replicates ± standard deviation; values marked with the same letter do not differ significantly.

The tested starch pastes were also subjected to rheological analysis. Due to the fact that the starch pastes are non-Newtonian fluids, the Ostwald de Waele model was used to describe their flow characteristics. The power law model used for the analysis is characterized by a base, which is defined as the consistency coefficient (K), and an exponent defined as the flow index (n), which determines the convergence with Newtonian flow. These parameters and the value of the area of thixotropic hysteresis loop for investigated oxidized potato starch pastes are presented in Table 4. The rheological properties studies confirmed earlier observations from the analysis of the gelatinization process and texture profile. Over time, the tested starches underwent depolymerization in the reactor, accompanied by a progressive decrease in the consistency coefficient (K) and an increase in convergence with Newtonian flow (n). At the same time, the area of the thixotropic hysteresis loop decreased. This change is desirable as thixotropy is considered unfavorable as it indicates a lack of

rheological stability of the tested material. Another similarity between previous tests and rheological properties concerns the sample subjected to oxidation for only one hour, for which an increase in viscosity was observed. This phenomenon was manifested by an increase in consistency index and decrease in flow index. Moreover, the aforementioned paste was most similar to the commercial preparation (LU 1404-1), similarly as in case of texture profile analysis.

Table 4. Flow characteristics of 5% potato starch pastes obtained from preparations subjected to different durations of ozone treatment.

Starch	Consistency Index ($Pa \cdot s^n$)	Flow Index (-)	Thixotropy ($Pa \cdot s^{-1}$)
Native	9.2 ± 0.1^{b}	0.656 ± 0.001^{bc}	$14{,}440 \pm 710^{c}$
Oxidized 1 h	11.6 ± 1.3^{b}	0.581 ± 0.006^{b}	$14{,}209 \pm 9010^{bc}$
Oxidized 2 h	9.1 ± 0.1^{b}	0.609 ± 0.011^{bc}	$10{,}110 \pm 6492^{abc}$
Oxidized 4 h	5.0 ± 1.4^{a}	0.654 ± 0.052^{bc}	1546 ± 1550^{ab}
Oxidized 6 h	3.2 ± 0.5^{a}	0.689 ± 0.020^{c}	1359 ± 775^{ab}
Commercial	10.3 ± 1.4^{b}	0.453 ± 0.073^{a}	9491 ± 2191^{bc}

Mean value of three replicates ± standard deviation; values marked with the same letter do not differ significantly.

The next stage of the research was an attempt to accelerate the starch depolymerization reaction by reducing the reactor volume by 10 times, and thus increase the retention time. Due to a reduction in reactor volume, sampling was reduced only to the longest ozonation period, i.e., 6 h. The modified starch preparations ozonated in the small system were then subjected to the same rheological analysis as the preparations in previous subsection. To facilitate a comparison of results, the following chapter repeats the results for native starch and starch ozonated for 6 h in the large system. The pasting process of the tested oxidized potato starch preparations in the various reactors are presented in Table 5.

Table 5. Pasting characteristics of 5% potato starch suspensions treated with ozone at different concentrations (variable medium retention).

Starch	Pasting Temperature (°C)	Maximum Viscosity (BU)	Breakdown (BU)	Setback (BU)	End of Holding Period Viscosity (BU)	Final Viscosity (BU)
Reference	61.9	1879	1303	506	570	1082
Slow retention	63.2	1176	933	284	243	528
Fast retention	62.6	940	875	1	65	67

Mean value of three replicates; differences below 0.2 °C or 30 BU are considered not significant.

Analysis of data obtained for pasting process confirmed that using a smaller circulation capacity, which provided a higher ozone concentration in the reaction system, resulted in a starch preparation with significantly different characteristics. In addition to a significant decrease in viscosity parameters during gelatinization, a change in swelling characteristics was observed. Although the preparation obtained in the small reactor still had a peak viscosity, its setback value was negligible (1 BU), indicating no increase in viscosity during the cooling stage. Similar observations were could be drawn from research on gelling starch (commercial oxidized starch preparation modified by NaOCl with carboxyl group content of 0.11%) in case of which pasting characteristics of 6% suspension had negligible setback and final viscosity below 100 BU [40]. The obtained pasting profile once again indicated a different outcome of oxidation using ozone, when compared to the preparations obtained using sodium hypochlorite.

Analysis of the universal texture profile of the starch paste obtained from the fast retention system also confirmed the theory of more rapid starch depolymerization (Table 6), which is manifested by a decrease in hardness, loss of adhesiveness, and gumminess. The elasticity value close to unity suggests properties similar to a Newtonian fluid, e.g., water, but the cohesion of the formulation was relatively low, suggesting a thixotropic structure. This was confirmed by analysis of the flow curves (Table 7). Although the absolute thixotropy value for the fast retention formulation was the lowest, it was relatively high when compared to the consistency index (K). This observation is consistent with the flow index (convergence with Newtonian flow), which for the fast retention preparation was similar to native starch and lower than for the slow retention preparation.

Table 6. Texture profile of 5% potato starch pastes obtained from preparations treated with ozone at different concentrations (variable medium retention).

Starch	Hardness (N)	Adhesiveness (N·s)	Cohesiveness (-)	Springiness (-)	Gumminess (N)
Reference	0.45 ± 0.02^{b}	-0.10 ± 0.02^{b}	0.73 ± 0.00^{c}	0.97 ± 0.00^{a}	0.33 ± 0.02^{c}
Slow retention	0.41 ± 0.03^{b}	-0.04 ± 0.02^{a}	0.70 ± 0.02^{b}	0.98 ± 0.01^{a}	0.29 ± 0.01^{b}
Fast retention	0.37 ± 0.01^{a}	0.00 ± 0.00^{a}	0.42 ± 0.00^{a}	1.00 ± 0.00^{b}	0.15 ± 0.00^{a}

Mean value of three replicates ± standard deviation; values marked with the same letter do not differ significantly.

Table 7. Flow characteristics of 5% potato starch pastes obtained from preparations treated with ozone at different concentrations (variable medium retention).

Starch	Consistency Index ($Pa \cdot s^n$)	Flow Index (-)	Thixotropy ($Pa \cdot s^{-1}$)
Reference	9.2 ± 0.1^{b}	0.656 ± 0.001^{a}	$14{,}440 \pm 710^{b}$
Slow retention	3.2 ± 0.5^{a}	0.689 ± 0.020^{a}	1359 ± 775^{a}
Fast retention	0.8 ± 0.1^{a}	0.653 ± 0.026^{a}	753 ± 637^{a}

Mean value of three replicates ± standard deviation; values marked with the same letter do not differ significantly.

3.2.2. Influence of Suspension Concentration

In most cases, starch modification is carried out in an aqueous suspension, and the mass ratio of the prepared suspensions fluctuates around 1:1. Increasing the proportion of starch in the suspension could lead to the formation of a shear-thickening liquid, which would hinder mixing and number of further technological operations. On the other hand, reducing the starch concentration in the suspension leads to a decrease in reaction efficiency. However, due to the design of the ozonation system, in particular the ozone generator with an injector system based on a Venturi nozzle, lower concentrations are desired to prevent nozzle clogging. For the above reason, the effect of the starch suspension concentration on the efficiency of the oxidation reaction was studied. To this end, a sample with increased concentration of 30% was prepared and the reaction was carried out using small buffer tank.

The experiment showed that the use of a higher concentration did not accelerate the reaction to such an extent that the use of higher concentrations (i.e., above 20%) would be justified. This was confirmed by the analysis of data for the course of gelatinization (Table 8), texture profile (Table 9) and flow curves (Table 10), where it was found that the preparation obtained during the reaction of a 30% suspension in a small circulation system was more similar in its properties to the preparation obtained in a large circulation than in a small one, but at a suspension concentration of 20%. Nevertheless, potato starch suspension at concentration of 30% can be effectively modified, but the increase in ozone generation efficiency should be considered.

Table 8. Pasting characteristics of 5% potato starch suspensions treated with ozone using different suspension concentrations.

Suspension Concentration	Pasting Temperature (°C)	Maximum Viscosity (BU)	Breakdown (BU)	Setback (BU)	End of Holding Period Viscosity (BU)	Final Viscosity (BU)
20%	62.6	940	875	1	65	67
30%	62.2	1456	1345	85	108	194

Mean value of three replicates; differences below 0.2 °C or 30 BU are considered not significant.

Table 9. Texture profile of 5% potato starch pastes obtained from preparations treated with ozone using different suspension concentrations.

Starch	Hardness (N)	Adhesiveness (N·s)	Cohesiveness (-)	Springiness (-)	Gumminess (N)
20%	0.37 ± 0.01^{a}	0.00 ± 0.00^{a}	0.42 ± 0.00^{a}	1.00 ± 0.00^{a}	0.15 ± 0.00^{a}
30%	0.42 ± 0.00^{b}	0.00 ± 0.00^{a}	0.71 ± 0.02^{b}	1.00 ± 0.02^{a}	0.30 ± 0.01^{b}

Mean value of three replicates ± standard deviation; values marked with the same letter do not differ significantly.

Table 10. Flow characteristics of 5% potato starch pastes obtained from preparations treated with ozone using different suspension concentrations.

Starch	Consistency Index ($Pa \cdot s^{n}$)	Flow Index (-)	Thixotropy ($Pa \cdot s^{-1}$)
20%	0.8 ± 0.1^{a}	0.653 ± 0.026^{a}	753 ± 637^{a}
30%	2.4 ± 0.5^{b}	0.604 ± 0.030^{a}	1490 ± 173^{a}

Mean value of three replicates ± standard deviation; values marked with the same letter do not differ significantly.

3.3. Rheological Studies of Pea Starch

To analyze the effect of pea starch oxidation time, a suspension at a concentration of 20% and two types of reactors were prepared, analogously to the previous section. For the reaction system with a large buffer tank, samples of the suspension were taken at 1 h, 2 h, 4 h, and 6 h intervals, in the amount of approximately 1 L.

3.3.1. Influence of Oxidation Time

The characteristics of the gelatinization process of the tested preparations of oxidized pea starch at different reaction times are presented in Table 11. The gelatinization process of pea starch was very restricted (typical swelling characteristics of legume starches), and the viscosity of the tested preparations increased progressively in each phase of the study. For this reason, the tested preparations did not exhibit a viscosity peak, and the value given in Table 10 is identical or close to the value of viscosity in the final phase of incubation period. The above observations are also related to the fact that the breakdown values were equal to zero. On the other hand, the examined pea starch preparations exhibited a significant increase in viscosity during the cooling phase, which is indicated by high setback values.

The oxidation process caused a slight change in the gelatinization temperature of the tested preparations, but without a clear trend and at a level that was insignificant from a technological point of view, with the extreme difference being only 0.2 °C (considered as the accuracy of Brabender method). The duration of ozone treatment caused a progressive decrease in maximum viscosity and end of holding period viscosity (from 396 BU to 263 BU). On the other hand, setback values increased substantially (approx. by 100 BU), which resulted in an increase in final viscosity. The only exception was observed for starch subjected to the longest oxidation process (6 h), whose final viscosity was slightly lower than that of the initial product. Above characteristics may be associated with sol–gel

transition of pea starch paste that was further enhanced by oxidation process. Nevertheless, with longer ozone treatment (6 h) the depolymerization had more pronounced effect.

Table 11. Pasting characteristics of 7.5% pea starch suspensions subjected to different durations of ozone treatment.

Starch	Pasting Temperature (°C)	Maximum Viscosity (BU)	Breakdown (BU)	Setback (BU)	End of Holding Period Viscosity (BU)	Final Viscosity (BU)
Native	75.8	396	0	792	396	1194
Oxidized 1 h	75.8	378	0	904	378	1287
Oxidized 2 h	76.0	356	0	884	356	1245
Oxidized 4 h	75.9	299	0	909	299	1212
Oxidized 6 h	75.8	263	0	918	263	1186

Mean value of three replicates; differences below 0.2 °C or 30 BU are considered not significant.

The obtained characteristics of pea starch preparations can provide added value in applications where low hot viscosity (which facilitates processing) and high cold viscosity (water binding, adhesion) are required. Those may include film-forming and construction material applications.

Analysis of the texture profile of oxidized pea starch pastes (Table 12) confirmed the hypothesis of increased viscosity of cold pea starch gels. The observed changes concerned increased hardness, adhesiveness, and gumminess. The parameters of cohesiveness and springiness remained at a similar level. The lack of a visible linear effect of oxidation time on the texture profile of pea starch pastes was most likely related to the strong and rapid gelation process, which leads to a large range of the raw data. In such cases, rheological methods (Table 13), which are more sensitive and can facilitate interpretation of the results. Analysis of the flow curves of pastes confirmed depolymerization of pea starch, manifested by a decrease in the consistency index and an increase in convergence with Newtonian flow. The only exception was observed for paste of starch modified for the shortest duration which had the highest consistency index 46.0 $Pa{\cdot}s^n$. These observations are consistent with pasting characteristic (highest final viscosity) and texture profile (highest hardness) of that paste. Moreover, mild oxidation (1–4 h) of pea starch led to an increase in anti-thixotopic behavior (indicated by the minus sign). This phenomenon, similar to thixotropy, is undesired and indicates a lack of rheological stability, as values close to 0 are considered favorable. Nevertheless, longer oxidation led to a decrease in anti-thixotopic behavior when compared to native starch.

Table 12. Texture profile of 7.5% pea starch pastes obtained from preparations subjected to different durations of ozone treatment.

Starch	Hardness (N)	Adhesiveness (N·s)	Cohesiveness (-)	Springiness (-)	Gumminess (N)
Native	2.96 ± 1.11^{a}	-9.16 ± 0.91^{a}	0.50 ± 0.05^{a}	0.97 ± 0.02^{a}	1.44 ± 0.40^{a}
Oxidized 1 h	3.61 ± 0.57^{a}	-14.67 ± 11.21^{a}	0.49 ± 0.03^{a}	0.97 ± 0.02^{a}	1.76 ± 0.38^{a}
Oxidized 2 h	3.38 ± 0.14^{a}	-14.86 ± 3.97^{a}	0.52 ± 0.02^{a}	0.93 ± 0.07^{a}	1.76 ± 0.02^{a}
Oxidized 4 h	3.48 ± 1.05^{a}	-11.07 ± 10.42^{a}	0.53 ± 0.03^{a}	0.97 ± 0.01^{a}	1.85 ± 0.73^{a}
Oxidized 6 h	3.11 ± 0.91^{a}	-13.12 ± 8.64^{a}	0.51 ± 0.03^{a}	0.93 ± 0.07^{a}	1.58 ± 0.37^{a}

Mean value of three replicates ± standard deviation; values marked with the same letter do not differ significantly.

Table 13. Flow characteristics of 7.5% pea starch pastes obtained from preparations subjected to different durations of ozone treatment.

Starch	Consistency Index ($Pa \cdot s^n$)	Flow Index (-)	Thixotropy ($Pa \cdot s^{-1}$)
Native	44.9 ± 2.9^{c}	0.263 ± 0.016^{a}	-5080 ± 4197^{ab}
Oxidized 1 h	46.0 ± 1.3^{c}	0.271 ± 0.029^{a}	$-12{,}651 \pm 4029^{bc}$
Oxidized 2 h	40.1 ± 0.9^{bc}	0.288 ± 0.004^{ab}	$-13{,}236 \pm 1846^{bc}$
Oxidized 4 h	37.0 ± 1.6^{ab}	0.326 ± 0.013^{bc}	$-16{,}505 \pm 5112^{c}$
Oxidized 6 h	30.8 ± 4.1^{a}	0.343 ± 0.033^{c}	-2206 ± 818^{a}

Mean value of three replicates ± standard deviation; values marked with the same letter do not differ significantly.

3.3.2. Influence of Retention Volume

The oxidation of pea starch was further carried out in a small reactor, and the tested ozonated starch preparations were subjected to the same rheological analysis. In order to facilitate the comparison of results, the results for native pea starch and ozonated pea starch for 6 h in a large circulation will be repeated in the following subsection. The gelatinization process of the tested preparations of oxidized pea starch in different reactors is presented in Table 14. The changes in pasting characteristics of pea starch obtained in small circulation system did not change as spectacularly as in the case of potato starch. Nevertheless, the phenomenon of reduced hot starch paste viscosity intensified, which in case of fast retention was only 140 BU. The final viscosity remained unchanged (what could be once again attributed to strong gelling properties).

Table 14. Pasting characteristics of 7.5% pea starch suspensions treated with ozone at different concentration (variable medium retention).

Starch	Pasting Temperature (°C)	Maximum Viscosity (BU)	Breakdown (BU)	Setback (BU)	End of Holding Period Viscosity (BU)	Final Viscosity (BU)
Reference	75.8	396	0	792	396	1194
Slow retention	75.8	263	0	918	263	1186
Fast retention	75.8	140	0	1061	140	1201

Mean value of three replicates; differences below 0.2 °C or 30 BU are considered not significant.

A similar observation regarding texture profile analysis of pea starch pastes as in the case of large circulation system applied also to small circulation tank (Table 15). Due to strong gelation phenomenon, the analysis did not provide clear confirmation pointing to more pronounced depolymerization of starch. Nevertheless, the hardness of the paste obtained from starch modified in the small circulation system (fast retention) was the lowest, this was also reflected in gumminess value.

Table 15. Texture profile of 7.5% pea starch pastes obtained from preparations treated with ozone at different concentrations (variable medium retention).

Starch	Hardness (N)	Adhesiveness (N·s)	Cohesiveness (-)	Springiness (-)	Gumminess (N)
Reference	2.96 ± 1.11^{a}	-9.16 ± 0.91^{a}	0.50 ± 0.05^{a}	0.97 ± 0.02^{a}	1.44 ± 0.40^{a}
Slow retention	3.11 ± 0.91^{a}	-13.12 ± 8.64^{a}	0.51 ± 0.03^{a}	0.93 ± 0.07^{a}	1.58 ± 0.37^{a}
Fast retention	2.62 ± 1.20^{a}	-11.35 ± 1.44^{a}	0.50 ± 0.05^{a}	0.92 ± 0.03^{a}	1.32 ± 0.38^{a}

Mean value of three replicates ± standard deviation; values marked with the same letter do not differ significantly.

Analysis of the flow characteristics of oxidized pea starch paste in a small circulation system (Table 16) confirmed a decrease in paste viscosity and an increase in convergence

with Newtonian flow in relation to both native and oxidized starch modified in a large circulation system. Considering relatively high final viscosity and the values of the Ostwald de Waele equation coefficients, it was reasonable to conduct further basic research on the oxidation of pea starch in order to deepen the understanding of the depolymerization and gelation mechanisms of those preparations.

Table 16. Flow characteristics of 7.5% pea starch pastes obtained from preparations treated with ozone at different concentrations (variable medium retention).

Starch	Consistency Index ($Pa \cdot s^n$)	Flow Index (-)	Thixotropy ($Pa \cdot s^{-1}$)
Reference	44.9 ± 2.9^{b}	0.263 ± 0.016^{a}	-5080 ± 4198^{a}
Slow retention	30.8 ± 4.1^{a}	0.343 ± 0.033^{b}	-2206 ± 818^{a}
Fast retention	25.9 ± 2.9^{a}	0.395 ± 0.011^{b}	-1742 ± 255^{a}

Mean value of three replicates ± standard deviation; values marked with the same letter do not differ significantly.

3.4. Molecular Mass Distribution and Hydrodynamic Parameters of Modified Starch Macromolecules

Apart from depolymerization, oxidation process with sodium hypochlorite is believed to be accompanied by increase in polydispersity of obtained starch preparations [19]. Those observations are also true for investigated ozonated starch preparations of both botanical sources (Tables 17 and 18). Generally, the changes in molecular mass distribution and hydrodynamic parameters of starch, as a result of the modification process, were consistent between both varieties and changed gradually with the extent of modification process. After treatment with ozone for 2 h the effect of depolymerization of starch was minor, with a decrease in number average molar weight, average molar weight, and z-average molar weight only by a fraction of the initial value. After four hours of treatment with ozone, the molecular masses decreased substantially for potato starch (approximately by half), whereas for pea starch this statement was only true for M_n. This indicates that in case of potato starch, the depolymerization process takes place at similar rate regardless of starch molecule size, whereas for pea starch smaller molecules are more prone towards modification. After modification for four hours, changes in polydispersity of the samples started to be evident. However, due to aforementioned mechanism of modification, it is more notable for pea starch. Changes in the molecular mass distribution at this time interval were also reflected in hydrodynamic parameters of starch macromolecules in the solution. The oxidation led to a decrease in hydrodynamic radius and radius of gyration, indicating smaller molecule size and their more compact shape in the solution. Those observations are also consistent with potato starch treated with sodium hypochlorite [40]. A further increase in the dose of ozone (6 h and fast retention) resulted in substantial depolymerization of both potato and pea starch, which eventually led to a reduction in M_n, M_w, and M_z by at least a level of magnitude. This was accompanied by a substantial increase in polysperisty index (approximately by five) and decrease in macromolecule radius by half. Those observations indicate that although pea starch is less prone to modification by ozone treatment than potato, increasing the dose of ozone will lead continuous depolymerization of both substrates.

Table 17. Molecular mass distribution and hydrodynamic parameters of potato starch macromolecules.

Starch	M_n (Da)	M_w (Da)	M_z (Da)	M_w/M_n	R_h (nm)	R_g (nm)
Native	$2.05 \times 10^7 \pm 2.19 \times 10^{6\,c}$	$4.09 \times 10^7 \pm 3.70 \times 10^{6\,b}$	$6.25 \times 10^7 \pm 7.49 \times 10^{6\,b}$	2.0 ± 0.2^{a}	99 ± 5^{b}	172 ± 12^{c}

Table 17. *Cont.*

Starch	M_n (Da)	M_w (Da)	M_z (Da)	M_w/M_n	R_h (nm)	R_g (nm)
Oxidized 2 h	$1.34 \times 10^7 \pm 1.22 \times 10^6$ b	$2.63 \times 10^7 \pm 1.85 \times 10^6$ a	$3.97 \times 10^7 \pm 1.62 \times 10^6$ a	2.0 ± 0.1 a	95 ± 1 b	149 ± 2 b
Oxidized 4 h	$1.04 \times 10^7 \pm 7.56 \times 10^5$ b	$2.33 \times 10^7 \pm 2.12 \times 10^6$ a	$3.65 \times 10^7 \pm 7.91 \times 10^5$ a	2.2 ± 0.0 a	90 ± 6 b	136 ± 4 b
Oxidized 6 h (FR)	$6.05 \times 10^5 \pm 6.47 \times 10^4$ a	$7.19 \times 10^6 \pm 1.64 \times 10^5$ a	$3.36 \times 10^7 \pm 2.48 \times 10^6$ a	12.0 ± 1.3 b	42 ± 5 a	92 ± 7 a

Mean value of three replicates ± standard deviation; values marked with the same letter do not differ significantly.

Table 18. Molecular mass distribution and hydrodynamic parameters of pea starch macromolecules.

Starch	M_n (Da)	M_w (Da)	M_z (Da)	M_w/M_n	R_h (nm)	R_g (nm)
Native	$2.10 \times 10^7 \pm 3.07 \times 10^5$ d	$6.64 \times 10^7 \pm 1.98 \times 10^6$ c	$1.23 \times 10^8 \pm 1.35 \times 10^6$ c	3.2 ± 0.0 a	83 ± 3 b	159 ± 2 c
Oxidized 2 h	$1.80 \times 10^7 \pm 1.57 \times 10^6$ c	$6.19 \times 10^7 \pm 1.32 \times 10^6$ c	$1.20 \times 10^8 \pm 4.22 \times 10^6$ c	3.5 ± 0.4 a	91 ± 5 b	149 ± 10 bc
Oxidized 4 h	$8.89 \times 10^6 \pm 4.26 \times 10^5$ b	$4.90 \times 10^7 \pm 2.57 \times 10^6$ b	$1.03 \times 10^8 \pm 2.60 \times 10^6$ b	5.5 ± 0.5 b	54 ± 5 a	138 ± 10 b
Oxidized 6 h (FR)	$1.44 \times 10^6 \pm 1.31 \times 10^5$ a	$1.56 \times 10^7 \pm 1.20 \times 10^6$ a	$1.67 \times 10^7 \pm 2.48 \times 10^6$ a	10.9 ± 0.8 c	49 ± 4 a	92 ± 5 a

Mean value of three replicates ± standard deviation; values marked with the same letter do not differ significantly.

3.5. Principal Component Analysis

In order to further explore similarities between rheological characteristics of obtained ozonated starch preparations, principal component analysis (PCA) was performed. The first two principal components presented on the PCA plots explain almost 90% of the total variance (Figures 1 and 2).

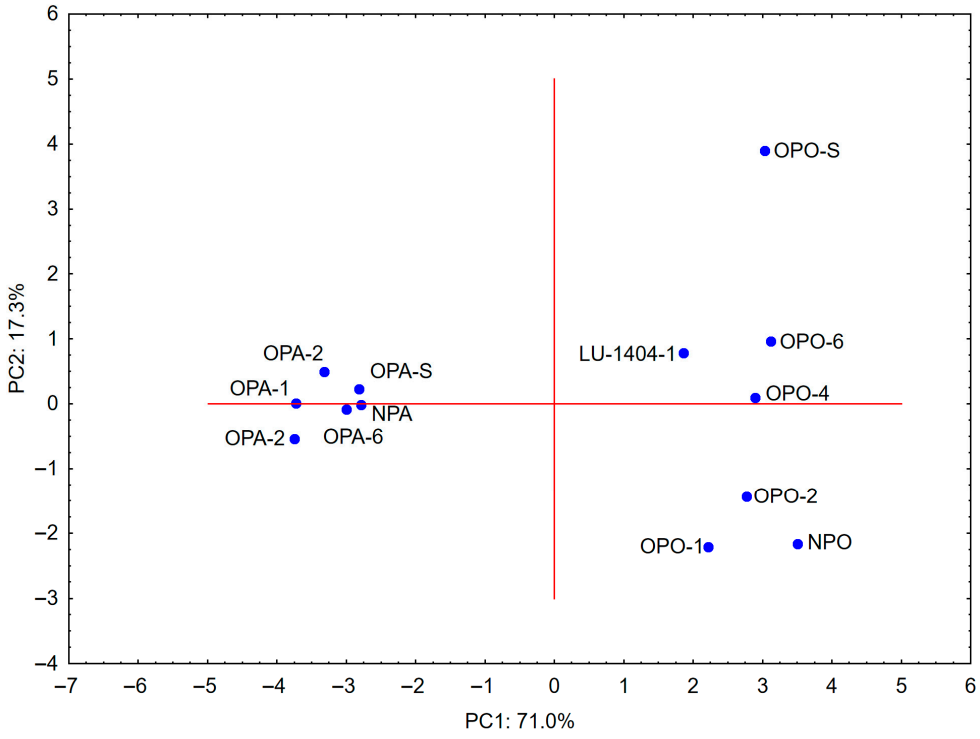

Figure 1. PCA score plot for rheological characteristics of ozone treated starches (data contained within Sections 3.2 and 3.3).

The objects presented on the score plot can be categorized into three clusters. The first group is characterized by negative PC1 values and PC2 close to 0. This group includes all pea starch preparations regardless of the extent of modification. This indicates that their rheological characteristic was only slightly affected by the ozonation process. Considering the PCA loadings plot, this phenomenon should be attributed to high Brabender final viscosity, setback, pasting temperature, consistency index, hardness, and gumminess. This

confirms previous conclusion regarding the major role of the gelling process in rheological characteristics of pea starch preparations. The second group of objects was characterized by positive PC1 values and negative PC2, it consisted of native potato starch and potato starch preparations subjected to shortest ozonation period (1 and 2 h). This group of starches was unique in terms of peak viscosity, end of incubation period viscosity, cohesiveness, and thixotropy. The last group included potato starch ozonated for 4 and 6 h, as well as commercial oxidized starch. This cluster was characterized by positive PC1 values and PC2 close to 0, indicating higher flow behavior index (convergence with Newtonian flow) and lower adhesiveness.

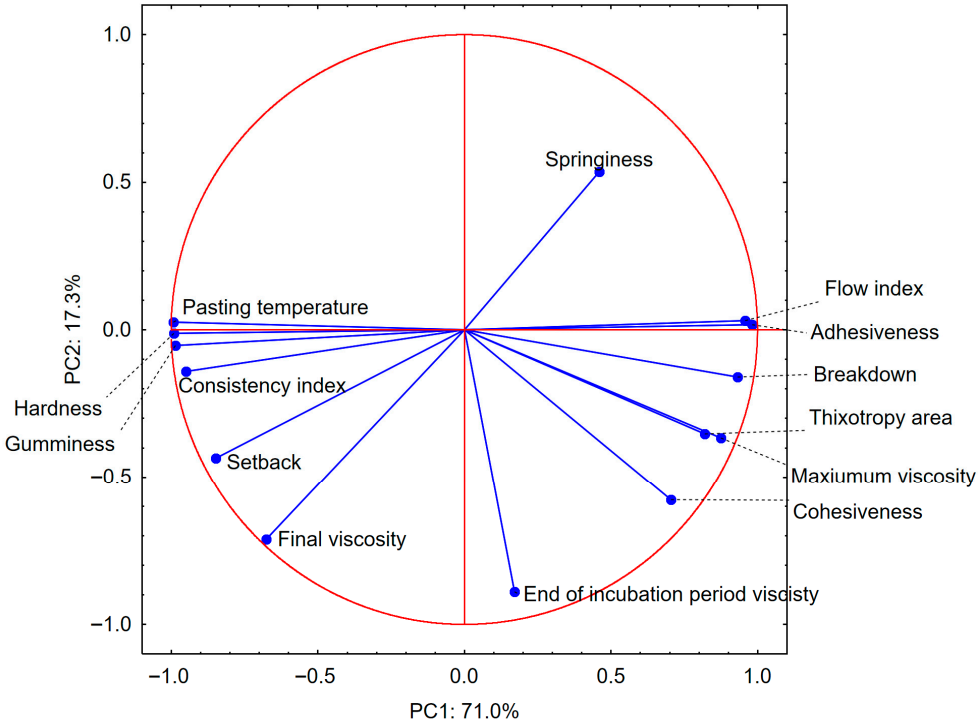

Figure 2. PCA loadings plot for data presented in Sections 3.2 and 3.3.

The PCA plots highlighted the differences between analyzed preparations and confirmed that with the increase in degree of oxidation of potato starch, rheological properties change gradually. Whereas for pea starch preparations those observations are only partially true, due to strong gelling phenomenon.

3.6. Research Limitations and Directions of Further Research

This research studied ozone treatment of starch at a pilot scale using a commercially available ozone generator with an emphasis on practical aspect of modification only (rheological properties). The studied technology is readily scalable by incorporation of available generators of ozonated water with higher outputs and by adjustment of auxiliary equipment (including pumps and buffer tanks, and by increasing diameters of hoses/pipelines). Nevertheless, inevitable alteration of the flow characteristics and ozone dosing in case of larger reactors may have a negative impact on the efficiency or mechanics of the modification process, and should be considered in scaling up of the technology. Moreover, the differences in environmental impact of modification by ozone vs. sodium hypochlorite should be considered. The advantages of ozone treatment include the lack of using toxic chemicals and possible water savings due to lack of necessity of washing residual reagents, moreover after filtration of starch subjected to ozone treatment, the technological water can be recirculated. Considering the above, starch ozonation can be considered as no waste

technology, as water is the only sidestream of the process. Nevertheless, the energy consumption required for oxygen concentration, ozone generation, and maintaining the flow in the system should be considered in life cycle assessment studies (LCA). It should noted that estimated energy consumption for the production of 1 kg of potato starch preparation with carboxyl group content of 0.33% (considered as moderate to extensive modification) will consume 2.25 kWh of electric energy in ozone generation. Moreover, considering the health and safety of the production process at the industrial scale, ozone monitoring and presumably scavenging should be considered, thus affecting the above assessment. Therefore, modification of starch by ozone treatment can be considered green, as being a zero-waste approach, but the overall environmental impact will be greatly affected by energy mix considered in the LCA study. Furthermore, although ozone is recognized as safe (GRAS) for direct contact with food products, it is not on the list of modifying agents that can be used for production of food grade oxidized starch preparations according to Joint FAO/WHO Expert Committee on Food Additives [15], which may be interpreted as a legal loophole. Considering the above further studies should consider monitoring safety for human consumption or at least free radicals formation.

4. Conclusions

It was found that the oxidation of potato starch with ozone in an aqueous suspension is an effective method for obtaining this type of starch preparation. At the same time, the degree of oxidation depends on the reaction time, and with the reaction time, a change in the gelatinization characteristics, texture profile, and rheological properties typical of the oxidation process using sodium hypochlorite is observed. At the same time, the results obtained for the commercial preparation LU1404-1 indicate a potentially different depolymerization mechanism.

On a pilot scale, the specific electrical input for ozone generation was approximately 2.25 kWh per kilogram of preparation with extent of modification measured as carboxyl group content reaching 0.33% for potato starch and 0.11% for pea. Overall environmental performance and off-gas handling remain installation dependent. Furthermore, the results of the study indicate that the rheological effects of starch ozonation are significantly more noticeable for potato starch than for pea starch, which was attributed to the strong gelatinization process of pea starch preparations and lower oxidation reaction efficiency, as indicated by carboxyl group content.

In a 20% aqueous suspension of potato starch, after 6 h of ozone treatment at 20 °C, the maximum viscosity was reduced to 1176 BU compared to 1879 BU for native starch, where after the incubation period the viscosity value decreased to 243 BU compared to 570 BU for native starch, and a setback value was 284 BU compared to 506 BU for native potato starch. In turn, for pea starch under the same conditions, the maximum viscosity was reduced to 263 BU, compared to 396 BU for native starch. After incubation, the viscosity decreased to 263 BU compared to 396 BU for native starch, and the setback index increased to 918 BU from 792 BU for native pea starch.

Gel permeation chromatography with triple detection confirmed continuous depolymerization of potato and pea starch by ozone treatment. This was reflected by reduction in molecular mass, accompanied by increase in polydispersity and decrease in macromolecule radius in the solution. During the most intensive ozone treatment in the study the molecular mass of starch, preparations decreased by a level of magnitude.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app152010924/s1, Figure S1: SEC chromatograms for potato starch, signals from the refractive index detector; Figure S2: SEC chromatograms for pea starch, signals from the refractive index detector; Document S3: Brabender pasting curves of potato and pea starch preparations.

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